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Synthesis, Characterization and Biological Activity Studies of 1,3,4-Oxadiazole Analogs

Bhat KI, Sufeera K, Chaitanya Sunil Kumar P

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte University, Deralakatte, Mangalore, Karnataka, India

Address for correspondence: *Dr. K Ishwar Bhat, E-mail: bhatishwar@yahoo.co.in*

ABSTRACT

The reaction of *p*-bromoanilino acetohydrazide(II) with aromatic aldehydes in alcohol yielded 2-[4-bromo aniline] N-substituted benzylidine hydrazides (IIIa-IIIj), which in presence of yellow mercuric oxide and iodine in DMF, yielded corresponding 4-bromo[(N-5-substituted 1,3,4 oxadiazole-2 –yl)methyl]aniline (IVa-IVj). Structures of the compounds synthesized were confirmed by IR, ^{1}HNMR and MASS spectroscopic analysis. The newly synthesized compounds were screened for antibacterial, antifungal and anti-inflammatory activities. Some of the compounds showed remarkable antibacterial, antifungal and anti-inflammatory activities.

Key words: 1,3,4-Oxadiazoles, antibacterial activity, antifungal activity, anti-inflammatory activity

INTRODUCTION

Oxadiazoles are five-membered heterocyclic compounds with two nitrogen and one oxygen atoms. They are synthesized by ring condensation and rearrangement reactions. Some of the recent studies have shown that 1,3,4-oxadiazoles and its derivatives were reported to possess antimicrobial,[1] anti-inflammatory,[2] antibacterial,[3] anticancer,[4] antifungal,[5] tuberculostatic[6] and analgesic[7] activities. Moreover the amino compounds are very commonly used as antimicrobial and germicidal drug. By considering all the above factors, it was thought to synthesize some substituted oxadiazoles derivatives derived from *p*-bromo aniline moiety. The compounds synthesized were screened for anti-inflammatory, antibacterial and antifungal activities.

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MATERIALS AND METHODS

All the melting points were determined by open capillary method and were uncorrected. The final products were purified by recrystallization. The IR spectra were recorded by PERKIN ELMER FT-IR Spectrophotometer using a thin film supported on KBr pellets. ^{1}HNMR spectra were obtained in DMSO and chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard. Mass spectra were recorded on Jeol SX 102/Da-600 mass spectrometer.

Synthesis of ethyl-2-(4-bromophenylamino) acetate (I)

A mixture of *p*-bromo aniline (13.9 g., 0.1 mol), ethylchloroacetate(12.25 ml, 0.1 mol) and anhydrous

potassium carbonate (19.5 g, 0.15 mol) in dry acetone was refluxed on a water bath for 24 hours at 70°C. The resultant reaction mixture was cooled and filtered. From the filtrate, excess of acetone was removed by distillation. The resultant solid was recrystallized from ethanol mp:150-152°C;

IR(KBr) cm^{-1}:3374 (NH), 1720(C = O), 1600(C = C) 2851 (CH),599 (C--Br).

^{1}H nmR(δ ppm): 7.16- 7.36 (4H, m,Ar),7.89 (1H,s,NH), 4.2-4.1 (2H,q,CH_2), 3.85 (2H, s, CH_2), 1.28-1.24 (2H,t,CH_3).

Synthesis of 2-(4-bromophenylamino) acetohydrazide (II)

A mixture of (I) (12.9 g.,0.05mol) and hydrazine hydrate (2.4 ml.,99%, 0.075mol) in ethanol (100 ml) was refluxed on a water bath for 6 hours. From the reaction mixture, excess of ethanol was removed by distillation. On cooling the resultant mixture, white needle like crystals of *p*-bromo anilino aceto hydrazide began to separate. It was collected, filtered and recrystallized from ethanol mp:184-186°C;

IR(KBr) cm−1:3374(NH str), 2902 (C-H), 1640 (C = O), 600(C--Br).1HNMR(δ ppm): 9.5(1H,s,CONH), 8.5 (1H,s,NH), 6.4-7.1 (4H,m,Ar),4.2-4.15 (2H,s,NH_2), 3.84 (2H,s,CH_2).

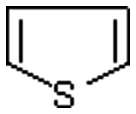
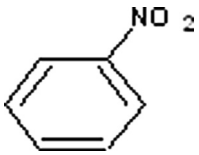
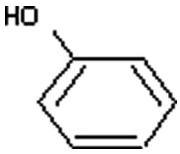
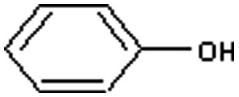
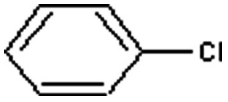
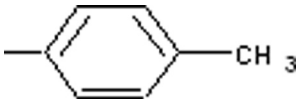
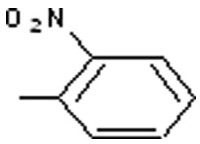
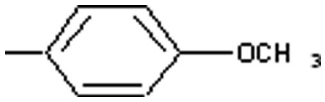
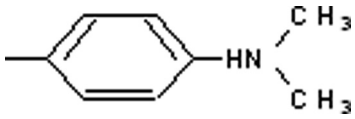
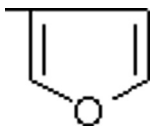
Synthesis of 2-(4-bromophenylamino)-N'- (substituted benzylidene) acetohydrazide(IIIa-IIIj)

A mixture of (II) (0.01 mol) and different aromatic or heterocyclic aldehyde (0.01 mol) in ethanol was refluxed in presence of few drops of glacial acetic acid in a round bottom flask on a water bath for 6 hours. The reaction mixture was cooled and the solid thus separated was filtered, washed with ice cold water and recrystallized from ethanol. Table 1 enlists various substituents and Table 2 summarizes physical data of the above compounds.

Synthesis of 4-bromo-N-((5-(substitutedphenyl)-1,3,4-oxadiazol-2-yl)methyl)aniline:(IVa-IVj)

A solution of Schiff base (IIIa-IIIj) (3.78 g.,0.01 mol(III b) in DMF (40 ml) was stirred in presence of yellow mercuric oxide (3 g) and iodine (1.5 g) at room temperature for 48 hr under anhydrous conditions. The reaction mixture was filtered and poured onto crushed ice and stirred well. The solid thus separated out was washed with water and recrystallized from DMF::ethanol (1:1). Table 3 summarizes the physical data of these compounds.

Table 1: Various substituents in titled compounds (IVa-IVj)

Compound	R
IIIa/IVa	
IIIb/IVb	
IIIc/IVc	
IIId/IVd	
IIIe/IVe	
IIIf/IVf	
IIIg/IVg	
IIIh/IVh	
IIIi/IVi	
IIIj/IVj	

IIId: 2-(4-Bromophenylamino)-N'-(4-hydroxybenzylidene) acetohydrazide

IR (KBr) cm^{-1}: 3374OH str,3292(NH str), 3111 (C-H str in CH_2), 1668,(C = O) str in amide,1601(C = C str), 502 (C--Br str).^{1}HNMR(δppm)9.82(1H,s,OH),9.78(1H,s, CONH),8.33(1H,s,NH),6.84-7.9(9H,mAr and N=CH),3.8-3.9(2H,s,CH_2).

Table 2: Physical data of compounds (IIIa-IIIj)

Compound	Purification solvent	Molecular formula	Molecular weight	Melting point (°C)	% yield
IIIa	Ethanol	$C_{13}H_{12}N_3OSBr$	339	224-226	65
IIIb	Ethanol	$C_{15}H_{13}N_4O_3Br$	378	215-217	60
IIIc	Ethanol	$C_{15}H_{14}N_3O_2Br$	349	230-232	65
IIId	Ethanol	$C_{15}H_{14}N_3O_3Br$	349	210-212	64
IIIe	Ethanol	$C_{15}H_{13}N_3OBrCl$	365	225-227	64
IIIf	Ethanol	$C_{16}H_{16}N_3OBr$	345	200-202	63
IIIg	Ethanol	$C_{15}H_{13}N_4O_3Br$	378	208-210	58
IIIh	Ethanol	$C_{16}H_{16}N_3O_2Br$	363	190-192	64
IIIi	Ethanol	$C_{17}H_{19}N_4OBr$	376	205-207	54
IIIj	Ethanol	$C_{13}H_{12}N_3O_2Br$	319	184-186	63

Table 3: Physical data of compound (IVa-IVj)

Compound	Purification solvent	Molecular formula	Molecular weight	Melting point (°C)	% yield
IVa	Ethanol:DMF (1:1)	$C_{13}H_{12}N_3OSBr$	335	204-206	60
IVb	Ethanol:DMF (1:1)	$C_{15}H_{11}N_4O_3Br$	376	195-200	56
IVc	Ethanol:DMF (1:1)	$C_{15}H_{12}O_2N_3Br$	345	240-242	54
IVd	Ethanol:DMF (1:1)	$C_{15}H_{12}N_3O_2Br$	345	245-247	60
IVe	Ethanol:DMF (1:1)	$C_{15}H_{11}N_3OBrCl$	365	238-239	59
IVf	Ethanol:DMF (1:1)	$C_{16}H_{14}N_3OBr$	343	230-232	62
IVg	Ethanol:DMF (1:1)	$C_{15}H_{11}N_4O_3Br$	376	235-237	65
IVh	Ethanol:DMF (1:1)	$C_{16}H1_4N_3O_2Br$	359	242-244	58
IVi	Ethanol:DMF (1:1)	$C_{17}H_{18}N_4OBr$	374	220-222	51
IVj	Ethanol:DMF (1:1)	$C_{13}H_{10}N_3O_2Br$	319	216-218	58

IVd:4-(5-((4-Bromophenylamino)methyl)-13,4-oxadiazol-2-yl)phenol

IR (KBr) cm−1: 3398(NH)3269, (OH),1602(C=N), 1589(C=C),1169 (C-O-C), 577 (C-Br).1H nmR(δ ppm):

10.36(1H,s,OH), 8.9(1H,s,NH),6.8--8.1(8H,m, Ar--H), 2.59 (2H,s,CH_2) Mass in m/z: Molecular ion peak was observed at m/z 345.

IV g.4-Bromo-N-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)aniline

IR (KBr) cm^{-1}**:** 3359(NH str), 1615(C=N str),1579(C=C str), 1158(C-O-C str),531(C-Br str)[1] H nmR(δ ppm):

8.37(1H, s, NH), 6.7--7.8(8H, m, Ar-H), 2.33(2H, s, CH_2) Mass in m/z: Molecular ion peak was observed at m/z 376.

IV h.4-Bromo-N-((5-(4-methoxy)--1,3,4-oxadiazol-2-yl)methyl)aniline

IR (KBr) cm^{-1}: 3289(NH str), 1600(C=N str),1596(C=C str), 1132(C-O-C str),562(C-Br str)[1] H nmR(δ ppm):

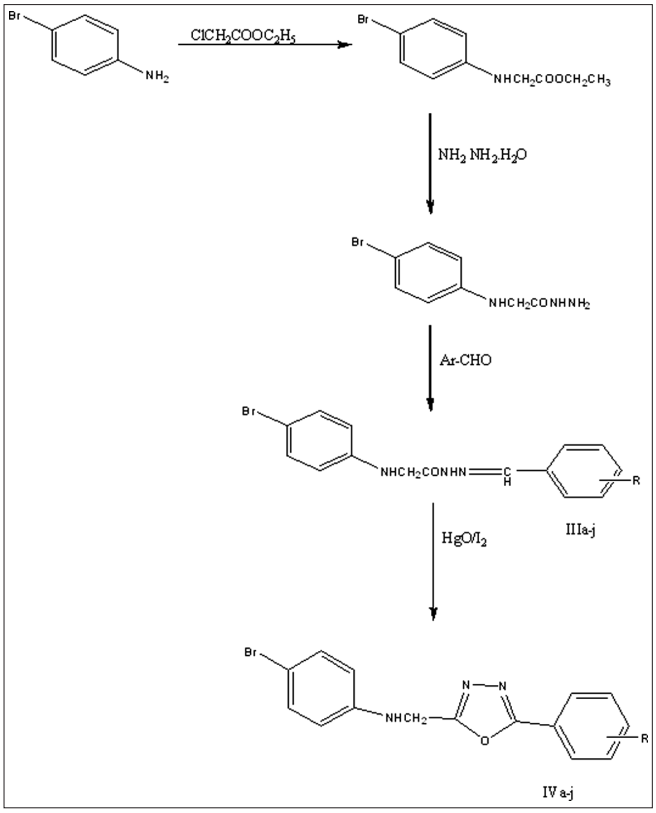
9.12(1H, s, NH),7.1-7.9(8H, m, Ar--H), 3.86 (3H,s, OCH_3), 2.56(2H, s, CH2) Mass in m/z: Molecular ion peak was observed at m/z 359.

RESULTS AND DISCUSSION

The main aim of this work was to synthesize various substituted 4-bromo [(N-5-substituted [1,3,4 -oxadiazole-2 –yl) methyl] aniline derivatives (Scheme 1). Initially N-substituted benzylidines (IIIa-IIIj) were synthesized by reacting p-bromoaceto hydrazide (II) with substituted aromatic aldehyde in ethanol. The titled compounds (IVa-IVj) were obtained by cyclization of N-substituted benzylidine hydrazide in presence of iodine and mercuric oxide in DMF.

All the synthesized compounds resulted in good yields with 50-65%. The formation of title compounds (IVa-IVj) is indicated by the disappearance of peak due to CH = N of the intermediate material Schiff base in IR and ^{1}H-NMR spectrum as given above. The IR of these compounds showed the presence of peaks due to (C=N and C-O-C) of the oxadiazole ring in all the compounds (IVa-IVj). The mass spectra of the title compounds are in confirmative with the assigned structure. The mass spectrum of these compounds showed molecular ion peaks corresponding to their molecular formula [Table 3].

Among the newly synthesized compounds IVb, IVc, IVd and IVg showed good antifungal activity. The electron withdrawing groups like chlorine and electron releasing groups like methoxy and methyl (IVe, IVf, IVh) showed



Scheme 1: (III a-j) 2-(4-bromophenylamino)-N'- (substituted benzylidene) acetohydrazide (IVa-j) 4-bromo-N-((5-(substituted phenyl)-1,3,4-oxadiazol-2-yl)methyl)aniline

Table 4: Antimicrobial activity of titled compounds (IVa-IVj)

Compound	Diameter of zone of inhibition (mm)				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
IVa	13	15	14	13	08
IVb	14	14	13	12	15
IVc	14	15	14	15	14
IVd	15	14	13	13	15
IVe	18	19	18	15	08
IVf	19	17	18	16	09
IVg	14	12	15	10	15
IVh	18	18	19	15	09
IVi	16	15	14	13	10
IVj	15	14	15	12	11
Amoxicillin	21	22	21	22	-
Ketoconazole	-	-	-	-	23

better antibacterial activity. Compounds like IVa, IVd, IVf, IVg and IVj showed moderate anti-inflammatory activity.

Biological evaluation

Antibacterial and antifungal activity

All the synthesized compounds were screened for their antibacterial activity[8] against *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis*, (MTCC 441), *Pseudomonas aeruginosa* (MTCC 1688) and *Escherichia coli* (MTCC 443) [*strain no. indicating the source of pure culture for all species used*] using amoxicillin as standard drug. All the synthesized compound were also screened for their antifungal activity against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282) using ketoconazole as standard drug. Compounds with substituents like – OH, – NO_2, [IVb, IVc, IVd, IVg] showed moderate antifungal activity. Compounds with substituents like *p*-OCH_3, *p*-Cl, *p*-CH_3, [IVe, IVf, IVh] showed better antibacterial activity. Data of antimicrobial activity is summarized in Table 4.

Anti-inflammatory activity by Carrageenan-induced rat hind paw edema method

Group I (Control)	Vehicle (p.o)
Group II (Standard)	Diclofenac 13.5mg/ kg body weight (p.o)
Group III to XII (Test)	40 mg/kg body weight (oral) of 1, 3, 4 oxadiazole derivatives in 0.6 %w/v DMSO

Animals were divided into 12 different groups contained six in each group. After 30 minutes of administration of standard and test compounds an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of 1% of carrageenan solution in the plantar tissue of the paw of all the animals. The initial paw volume was measured plethysmographically (IITC digital plethysmograph, IITC-520) within 30 second of the injection. The relative increase in paw volume was measured in control, standard and test groups at 1, 2, 3 and 4-hour intervals after carrageenan injection.[9]

The percentage inhibition of edema volume was calculated by using the formula

$$\text{Percentage inhibition} = \left(1 - \frac{V_t}{V_c}\right) \times 100$$

Where V_t and V_C are the relative change in the edema volume of paw after the administration of the test and control, respectively. Data of anti-inflammatory activity is summarized in Table 5.

Statistical analysis

All the values were expressed as mean ± SEM using One-way ANOVA followed by Dunnet's —*t* test.

CONCLUSION

The 1,3,4 oxadiazole derivatives reported showed good

Table 5: Anti-inflammatory activity of titled compounds (IVa-IVj)

Treatment	Dose mg/kg	Increase in paw volume (in ml)			
		1h	2h	3h	4h
Control	_	0.36 ± 0.06	0.68 ± 0.05	0.77 ± 0.03	0.81 ± 0.03
Diclofenac Sodium	13.5	0.18 ± 0.03* (50)	0.35 ± 0.05* (48.52)	0.41 ± 0.04* (46.75)	0.45 ± 0.05* (44.44)
IVa	40	0.20 ± 0.03* (44.44)	0.37 ± 0.03* (45.58)	0.42 ± 0.03* (45.45)	0.46 ± 0.03* (43.20)
IVb	40	0.23 ± 0.04 (36.11)	0.41 ± 0.04 (39.70)	0.47 ± 0.04 (38.96)	0.52 ± 0.04 (35.80)
IVc	40	0.24 ± 0.03 (33.33)	0.42 ± 0.02 (38.23)	0.46 ± 0.03 (40.25)	0.52 ± 0.03 (35.80)
IVd	40	0.19 ± 0.04* (47.22)	0.37 ± 0.03* (45.58)	0.42 ± 0.03* (45.45)	0.46 ± 0.04* (43.20)
IVe	40	0.19 ± 0.04* (47.22)	0.36 ± 0.03* (47.05)	0.41 ± 0.03* (46.75)	0.48 ± 0.03* (40.74)
IVf	40	0.20 ± 0.03* (44.44)	0.37 ± 0.03* (45.58)	0.42 ± 0.03* (45.45)	0.46 ± 0.03* (43.20)
IVg	40	0.23 ± 0.04* (47.22)	0.36 ± 0.03* (47.05)	0.45 ± 0.03* (46.75)	0.47 ± 0.03* (40.74)
IVh	40	0.16 ± 0.04 (17.77)	0.43 ± 0.04 (36.76)	0.50 ± 0.04 (35.06)	0.56 ± 0.03 (30.86)
IVi	40	0.23 ± 0.03 (36.11)	0.40 ± 0.03 (41.17)	0.47 ± 0.03 (38.96)	0.51 ± 0.04 (37.03)
IVj	40	0.22 ± 0.06 (38.88)	0.38 ± 0.03 (44.11)	0.45 ± 0.02 (41.55)	0.48 ± 0.04* (40.74)

All values are expressed as mean±SEM (*n=6*) **P*<0.05 significant compared to control

antimicrobial and anti-inflammatory activities. Derivatives IVb, IVc, IVd, IVg showed moderate antifungal activity. Compounds IVe, IVf, IVh, respectively, showed better antibacterial activity, whereas all the compounds showed good anti-inflammatory activity.

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